

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2433	2434	2435	2436	2437	2438	2439	2440	2441	2442	2
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(a) bringing into contact

a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, wherein the first and second recombination sites are variant recombination sites, and

(b) determining if recombination occurs between the first and second recombination sites, and determining if recombination occurs between the third and fourth recombination sites,

wherein recombination between the first and second recombination sites indicates that the mutant recombinase is a variant recombinase that mediates recombination at variant recombination sites,

wherein recombination between the third and fourth recombination sites indicates that the mutant recombinase retains the ability to mediate recombination at non-variant recombination sites.

2. The method of claim 1 wherein the recombination sites comprise recognition sequences and compatibility sequences,

wherein the recognition sequences of the first and second recombination sites differ from the recognition sequences of the third and fourth recombination sites,

wherein the compatibility sequences of the first and second recombination sites are sufficiently similar to allow recombination between the first and second recombination sites, and wherein the compatibility sequences of the third and fourth recombination sites are sufficiently similar to allow recombination between the third and fourth recombination sites, and

wherein the compatibility sequences of the first and second recombination sites differ from the compatibility sequences of the third and fourth recombination sites such that neither the first nor the second recombination site can be recombined with either the third or the fourth recombination site.

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3. The method of claim 1 wherein the first and second recombination sites cannot be recombined by non-mutant recombinase to a significant extent.

4. The method of claim 1 or 2 wherein the first and second recombination sites have identical sequences, and wherein the third and fourth recombination sites have identical sequences.

5. The method of claim 1 wherein recombination between the first and second recombination sites alters the expression of the first reporter gene, wherein recombination between the first and second recombination sites is determined by determining if expression of the first reporter gene is altered, and

wherein recombination between the third and fourth recombination sites alters the expression of the second reporter gene, wherein recombination between the third and fourth recombination sites is determined by determining if expression of the second reporter gene is altered.

6. The method of claim 5 wherein recombination between the first and second recombination sites allows the first reporter gene to be expressed.

7. The method of claim 6 wherein the first nucleic acid sequence further comprises a spacer sequence flanked by the first and second recombination sites, wherein the spacer sequence interrupts the first reporter gene such that the first reporter gene is not expressed, wherein recombination of the first and second recombination sites excises the spacer sequence which allows the first reporter gene to be expressed.

8. The method of claim 6 wherein a portion of the first reporter gene is inverted, wherein the inverted portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the inverted portion of the first reporter gene which allows the first reporter gene to be expressed.

9. The method of claim 5 wherein recombination between the first and second recombination sites prevents expression of the first reporter gene.

10. The method of claim 9 wherein the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites excises the first reporter gene which prevents expression of the first reporter gene.

11. The method of claim 9 wherein a portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the flanked portion of the first reporter gene which prevents expression of the first reporter gene.

12. The method of claim 5 wherein recombination between the third and fourth recombination sites allows the second reporter gene to be expressed.

13. The method of claim 12 wherein the second nucleic acid sequence further comprises a spacer sequence flanked by the third and fourth recombination sites, wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not expressed, wherein recombination of the third and fourth recombination sites excises the spacer sequence which allows the second reporter gene to be expressed.

14. The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not transcribed.

15. The method of claim 13 wherein the second reporter gene encodes a protein, wherein the spacer sequence interrupts the second reporter gene such that the protein encoded by the second reporter gene is not translated.

16. The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene produces an inactive expression product.

17. The method of claim 12 wherein a portion of the second reporter gene is inverted, wherein the inverted portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth

recombination sites inverts the inverted portion of the second reporter gene which allows the second reporter gene to be expressed.

18. The method of claim 5 wherein recombination between the third and fourth recombination sites prevents expression of the second reporter gene to be expressed.

19. The method of claim 18 wherein the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites excises the second reporter gene which prevents expression of the second reporter gene.

20. The method of claim 18 wherein a portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites inverts the flanked portion of the second reporter gene which prevents expression of the second reporter gene.

21. The method of claim 1 wherein the first nucleic acid sequence is a first nucleic acid construct and the second nucleic acid sequence is on a second nucleic acid construct.

22. The method of claim 21 wherein the first nucleic acid construct is an extrachromosomal vector and the second nucleic acid construct is in the genome of a host cell.

23. The method of claim 1 wherein the first and second nucleic acid constructs are on the same nucleic acid construct.

24. A method for producing site-specific recombination of DNA, comprising,

contacting a variant recombinase identified by the method of claim 1 with first and second DNA sequences,

wherein the first DNA sequence comprises a first recombination site and the second DNA sequence comprises a second recombination site,

wherein the variant recombinase mediates recombination between the first and second recombination sites thereby producing the site specific recombination.

25. The method of claim 24 wherein the first recombination site, the second recombination site, or both, are variant recombination sites.

26. The method of claim 24, wherein the first and second DNA sequences are connected by a pre-selected DNA segment.

27. The method of claim 26, wherein the first and second recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

28. The method of claim 27, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

29. The method of claim 27 further comprising contacting the variant recombinase with a fourth DNA sequence comprising a third recombination site, wherein the second and fourth DNA sequences are connected by a second pre-selected DNA segment.

30. The method of claim 29 wherein the first recombination site is a variant recombination site recognized by the variant recombinase and not by wild type recombinase, and wherein the second and third recombination sites are recombination sites recognized by wild type recombinase and by the variant recombinase.

31. The method of claim 30 further comprising, prior to contacting the variant recombinase with the first, second, and third recombination sites, contacting the recombination sites with wild type recombinase, thereby producing site specific recombination between the second and third recombination sites resulting in a deletion of the second pre-selected DNA segment.

32. The method of claim 29, wherein the second pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

33. The method of claim 26 wherein the first and second recombination sites have opposite orientations and the site-specific recombination is an inversion of the nucleotide sequence of the pre-selected DNA segment.

34. The method of claim 33, wherein the first and second recombination sites are variant recombination sites recognized by the variant recombinase.

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35. The method of claim 33, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

Sub 4 36. The method of claim 24, wherein the second and third DNA sequences are introduced into two different DNA molecules and the site-specific recombination is a reciprocal exchange of DNA segments proximate to the recombination sites.

37. The method of claim 36, wherein the first and second recombination sites are variant recombination sites recognized by the variant recombinase.

38. The method of claim 24 wherein the second DNA sequence includes a label, wherein recombination between the first and second recombination sites associates the label with the first DNA sequence.

39. The method of claim 38 wherein the first DNA sequence is a large circular DNA molecule.

Sub 5 40. The method of claim 24 wherein recombination occurs in a cell.

41. The method of claim 40 wherein the variant recombinase is contacted with the first and second DNA sequences by introducing into the cell a third DNA sequence comprising DNA encoding the variant recombinase.

42. The method of claim 41, wherein the third DNA sequence further comprises a regulatory nucleotide sequence and expression of the variant recombinase is produced by activating the regulatory nucleotide sequence.

43. The method of claim 40, wherein the cell is a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism.

44. The method of claim 43 wherein the multicellular organism is a plant, an animal, or a mammal.

Sub 6 45. The method of claim 40, wherein the first and second DNA sequences are connected by a pre-selected DNA segment, wherein the first and second recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

46. The method of claim 45 wherein the cell is in a multicellular organism.

47. The method of claim 45, wherein the pre-selected segment is an undesired marker or trait gene.

48. The method of claim 24 wherein the variant recombinase is contacted with the recombination sites *in vitro*.

49. The method of claim 48 wherein the method further comprises introducing the recombined DNA into a cell.

50. A method for cloning large DNA fragments, the method comprising concatenating DNA fragments to be cloned with vector arms, wherein each vector arm comprises a recombination site, wherein the DNA fragments and vector arms alternate in the concatemer,

introducing the concatemer into a cell expressing a variant recombinase identified by the method of claim 1, wherein the recombinase mediates recombination of the recombination sites thereby generating circles each containing a DNA fragment and a vector arm.

51. A method for cloning large DNA fragments, the method comprising ligating a DNA fragment to be cloned to vector arms, wherein each vector arm comprises (i) a blunt end, (ii) another end which is compatible with an end of the DNA fragment to be cloned, and (iii) a recombination site, wherein concatemers are not formed, and

introducing the ligated DNA fragment and vector arms into a cell expressing a variant recombinase identified by the method of claim 1.

52. A method for cloning large DNA fragments, the method comprising ligating a plurality of DNA fragments to be cloned with a plurality of first and second vector arms, wherein each first vector arm comprises two ligatable ends, wherein each second vector arm comprises a recombination site and one non-ligatable end,

wherein, following ligation, the DNA fragments and first vector arms alternate in concatemers, wherein the concatemers are flanked by second vector arms,

introducing the concatemers into a cell expressing a variant recombinase identified by the method of claim 1, wherein the recombinase mediates recombination of the recombination sites thereby generating circles containing the DNA fragments.

53. A variant recombinase identified by the method of claim 1.

54. A nucleic acid molecule encoding a variant recombinase identified by the method of claim 1.

55. The nucleic acid molecule of claim 54 wherein the nucleic acid molecule is a plasmid.

56. A cell containing the nucleic acid molecule of claim 54.

57. The cell of claim 56 wherein the cell is a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism.

58. The cell of claim 57 wherein the multicellular organism is a plant, an animal, or a mammal.

59. A nucleic acid molecule having at least one variant recombination site, wherein the variant recombination site is recognized by a variant recombinase identified by the method of claim 1 and is not recognized by wild type recombinase.

60. The nucleic acid molecule of claim 59 wherein the recombination site is not recognized by wild type recombinase.

61. The nucleic acid molecule of claim 59, wherein a first recombination site and a second recombination site are connected by a pre-selected DNA segment.

62. The nucleic acid molecule of claim 59 wherein the nucleic acid molecule is a plasmid.

63. A cell containing the nucleic acid molecule of claim 59.